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The Sensory Properties, Color, Microbial, Lipid Oxidation, and Residual Nitrite of *Se'i* Marinated with Lime and Roselle Calyces Extracts

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ABSTRACT

Meat deterioration can occur because of lipid oxidation and bacteria that could affect meat quality. It has been recognized that fruits of lime (*Citrus aurantifolia)* and roselle (*Hibiscus sabdariffa*) calyces contain bioactive compounds that have a capability to prevent oxidation and bacterial growth. The objective of this research was to investigate the effect of lime and roselle calyces extracts on *se'i* (Rotenese smoked beef) quality. Completely randomized design (CRD) with 2x4 factorial pattern was used in this study. The first factor (E) was source of extracts i.e., lime extract (E_1) and roselle extract (E_2). The second factor (L) was level of the extract consisted of 4 levels i.e., control (without extract/ L_0); L_1= 1%; L_2= 2%; and L_3= 3% (v/v). Each treatment consisted of 3 replications. Sensory properties measured were aroma, taste, and tenderness. Other variables measured were color, total plate count (TPC), thiobarbituric acid reactive substances (TBARS), and residual nitrite. The taste and tenderness of se'i were affected (P<0.05) by combination of the extract and the level of the extract. Results showed that there were significant interactions (P<0.05) between the kind of extracts and the level of extract on L (lightness), a (redness), and b (yellowness) values, TPC, TBARS, and residual nitrite values. The level of 3% of lime extract as well as 3% of roselle calyces extract improved score of taste and tenderness, reduced a values, decreased TPC, TBARS, and residual nitrite values. Marinating in 3% of roselle calyces extract decreased the b value but marinating in 3% of lime increased the b value of se'i. It is concluded that marinating 3% of roselle or 3% of lime gives the best effect on taste, tenderness, TPC, and TBARS values of *se'i*.

Keywords: roselle, lime, se'i, residual nitrite, sensory properties

ABSTRAK

Kerusakan pada daging disebabkan oleh terjadinya oksidasi lemak dan juga karena bakteri yang mempengaruhi kualitas daging. Telah dilaporkan bahwa dalam buah jeruk nipis dan kelopak bunga rosela terdapat komponen-komponen biooaktif yang mempunyai kemampuan menekan laju oksidasi lemak dan pertumbuhan bakteri. Tujuan penelitian ini adalah untuk menguji pengaruh ekstrak jeruk nipis dan ekstrak kelopak bunga rosela pada kualitas daging *se'i*. Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap 2 x 4 dengan pola faktorial. Faktor pertama adalah jenis ekstrak yang terdiri atas dua level, yaitu ekstrak jeruk nipis dan ekstrak kelopak bunga rosela. Faktor kedua adalah level pemberian yang terdiri atas 4 level, yaitu 0% (kontrol), 1%, 2%, 3% (v/v). Setiap perlakuan terdiri atas 3 ulangan. Peubah yang diukur adalah aroma, rasa, keempukan, warna, *total plate count* (TPC), *thiobarbituric acid reactive substances* (TBARS), dan residu nitrit. Hasil penelitian menunjukkan bahwa terdapat pengaruh interaksi antara jenis ekstrak dan level pemberian ekstrak pada rasa, keempukan, nilai *L* (*lightness*), *a* (*redness*), and *b* (*yellowness*), TPC, TBARS, dan residu nitrit s*e'i.* Level pemberian ekstrak jeruk nipis 3% atau ekstrak rosela 3% memberikan hasil tertinggi pada skor rasa dan keempukan, nilai terendah pada nilai *a*, TPC, TBARS, dan residu nitrit *se'i*. Level pemberian 3% ekstrak kelopak bunga rosela menurunkan nilai *b*, tetapi level pemberian 3% ekstrak jeruk nipis meningkatkan nilai *b*. Dapat disimpulkan bahwa penggunaan ekstrak jeruk nipis atau ekstrak kelopak bunga rosela dengan level 3% pada daging *se'i* memberikan pengaruh terbaik pada rasa, keempukan, TPC, dan TBARS.

Kata kunci: rosela, jeruk nipis, se'i, residu nitrit, sifat sensori

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INTRODUCTION

Generally, lipid in meat consists of triglycerides, phospholipids, cholesterols, and fat-soluble vitamins. Fatty acids in triglycerides consisted of both saturated and unsaturated fatty acids. When beef meat is stored, the double bond of unsaturated fatty acids changes to short chain fatty acids, aldehides, or ketones causing rancidity (Santos-Fandila *et al*., 2014). The process of lipid oxidation occur both in cooked and uncooked meats (El-Alim *et al*., 1999). Thus, in meat processing, antioxidant is usually added in order to reduce the rate of lipid oxidation (Shah *et al*., 2014).

Meat deterioration can occur because of bacteria. Meat is a nutrient–rich medium that offers ideal conditions for bacterial growth. The bacteria release their metabolites on meat surface during storage time causing organoleptic change (Dainty *et al*., 1985). Thus, the use of antibacterial agent is necessary in meat processing.

Antioxidant and synthetic antimicrobial such as butylated hydroxyl toluene (BHT) and nitrate have been used in meat industry for many years (Fernandez-Lopez *et al*., 2005). However, nowadays consumers are more concern about the negative impact of using synthetic additives on their health dealing with risk of toxicity and carcinogenicity (Ardabili *et al*., 2010). Thus, using natural additives such as plants to substitute the synthetic additive has notably increased (Sebranek *et al*., 2005; Ghafar *et al*., 2010). Such plants are lime (Kumari et *al*., 2013; Boshtam *et al*., 2011; Fernandez-Lopez *et al*., 2005) and rossele (Mahadevan *et al*., 2009; Karabacak & Bozkurt, 2008; Bozkurt & Belibagl, 2009).

Lime fruit contains flavonoids and vitamin C. Lime could decrease rancidity as many as 50% in beef meatballs (Fernandez-Lopez *et al*., 2005), reduced the number of total bacteria and residual nitrite of *se'i* (Malelak *et al*., 2015) and also could effectively tenderize beef and goat meats (Iqbal *et al*., 2016). These studies showed the beneficial function of lime in meat industry including antioxidant and antibacterial activities.

The effectiveness of roselle in reducing lipid oxidation (TBARS value) in sucuk (a vegetarian dry-fermented sausage) has been reported by Karabacak & Bozkurt (2008) and in kavurma (Tukey's traditional meat product) (Bozkurt & Belibagl, 2009). Moreover, addition of rosella in Sucuk can enhance the color, flavor, and texture of sucuk (Karabacak & Bozkurt, 2008). The addition of 1.5%-6% of dried roselle calyces in broiler diets reduced bacterial number both in fresh and cooked chicken meats (Onibi & Osho, 2007).

Se'i (Rotenesse smoked meat) is usually made from beef, sliced into rope-shape, spiced with salt and saltpeter, then it is smoked. Saltpeter has a function as a preservative particularly against *Clostridium botulinium* and give a red color to the product. In spite of these benefits, sometimes, addition of high amount of saltpeter in meat product can increase the level of nitrite residue in meat products. It was reported that high level of nitrite residue could bring the potential risk of childhood leukemia, brain tumors, and colon-rectal cancer (Demeyer *et al*., 2008). It was reported that citrus fruits juice containing organic acids had an ability to reduce the concentration of residual nitrite in cured meat products (Viuda-Martos *et al*., 2009). Organic acids are also found in roselle calyces such as: citric acid, ascorbic acid, maleic acid, malic acid, oxalic acid, and tartaric acid (Mahadevan *et al*., 2009). Addition of roselle or lime extract in other meat products such as *se'i*, could also affect their characteristics. The objectives of this experiment were to investigate the effect of lime and roselle calyces extract on the aroma, taste, tenderness, color, total plate count (TPC), thiobarbituric acid reactive substances (TBARS), and residual nitrite of *se'i*.

MATERIALS AND METHODS

Materials

The amount of 18 kg of beef meat obtained from butt and rump of Bali cattle cows were purchased from meat shop in Kupang, West Timor. Fresh roselle calyces and lime fruits were obtained from Oefafi Village, Kupang District. Calyces of roselle were dried in hot air oven at 60^{o}C for 3 d with 10.83% dry matter weight. The dried calyces were milled using laboratory hammer mill to obtain dry powder (1 mm mesh). To obtain 1%, 2%, and 3% (w/v) of *roselle* extract, each of 1, 2, and 3 g of the powder were poured into 350 mL volumetric glass and then distilled water were added up to 100 mL. The mixture was then shaked using an orbital shaker (Unimax 1010, Heidolph, Germany) for 2 h at room temperature, then filtered through a filter paper (Whatman no.4) (Mohd-Esa *et al*., 2010). The extract was stored at 4^{o}C for 2 d.

The limes were hammered and then sliced around from the top to the bottom and then squeezed to obtain the extract. The lime extract was put in jar glass and then distilled water was added up to 100 mL then the mixture was mixed thoroughly. The mixture was boiled in hot plate at 60^{o}C for 1 h and then filtered with Whatman (No.41) filter paper (Karabacak & Bozkurt, 2008) with a modification. To obtain 1%, 2%, and 3% (v/v) of the lime extract, each of 1, 2, and 3 mL of the extract were poured into 350 mL volumetric glass and then distilled water was added to reach the volume of 100 mL. Then the mixture was heated at 45^{o}C in a stirring hot plate for 30 min then filtered with Whatman (No.41) filter paper and stored at 4^{o}C for 2 d.

The calyces were dried in an oven at temperature of 60^{o}C for 3 d, and blended with Philips blender to obtain the mass. To obtain 1%, 2%, and 3% (v/v) of roselle extract, the amount of 1, 2, and 3 g of roselle mass were poured into volumetric glass and distilled water was added up to 100 mL, stirred at 60^{o}C for 5 min and filtered with Whatman (No.41) (Karabacak & Bozkurt, 2008) with a modification.

Experimental Design

Completely randomized design (CRD) with 2x4 factorial arrangment was used in this study. The first factor (E) was source of extracts consisted of 2 levels i.e., the lime extract (E_1) and the roselle extract (E_2). The second factor (L) was the level of the extract consisted of 4 levels i.e., control (without extract/ L_0); L_1= 1%; L_2= 2%; and L_3= 3%. Each treatment consisted of 3 replications.

Se'i Processing

Beef sample of 8500 g weight was trimmed off connective tissue and fat then sliced in rope-shape (*lalolak*) and then 2550 mg (300 mg/kg) saltpeter and 160 g salt were added and mixed well manually. Then, the beef was divided into 3 groups. The first group was control (without treatment). The second group was beef treated with the lime extract at doses of 1%, 2%, and 3% of the extract (v/v). The third group was beef treated with the roselle extract at doses of 1%, 2%, and 3% (v/v). Each group was well mixed and marinated for ± 12 h.

After marinating, the slices of beef were managed in frame then smoked using *Schleichera oleosa* wood and all meat surfaces were covered with *Schleichera oleosa* leaves while smoking. After smoking, *se'i* was placed and packed into a transparent 0.05 mm polyethylene plastic. The packaged sample was coded according to each treatment. The samples were then stored in a refrigerator at 4^{o}C before being analyzed.

Sensory Evaluation

Two training sessions were conducted for a twenty-five panelists from Animal Science Faculty students in order to familiarize them with the characteristics of *se'i* to be evaluated. To examine the aroma, 30 g of samples were sliced, put into small glass jars, and allowed to stand for several hours. One minute after opening the jars, the panelists immediately examined the aroma. The aroma characteristics were evaluated using 1-5 points category scale where one was odorless and 5 was very strong *se'i* aroma. For each replication from the treatments, the aroma was measured twice.

The taste and tenderness characteristics of the treated *se'i* were evaluated using a 1 to 9 scale (9= extremely like; 1= extremely dislike) and tenderness characteristics (9= extremely tender; 1= extremely tough). Each panelist had two pieces of *se'i* (± 2 mm thick x 2 cm length) for each of 3 replicates of each treatment. The samples were put in the white plate that had been labeled with three digit random numbers then the plates were offered randomly to the panelists. An average of the three measurements was used to calculate means score for each of treatment for the taste and tenderness of *se'i* sample.

Color Determination

Color determinations of *se'i* sample were applied in three measurements to calculate average scores for L, a, and b values. Color values were described as coordinates (lightness (L, black-white (darkness-lightness)), redness (a, ± red-green), and yellowness (b, ± yellow-blue). The color values were measured using Minolta colorimetry equipment (CR-200; Minolta Co), Osaka-Japan.

Microbial Analyses

Ten grams finely chopped of *se'i* from each treatment were homogenized with 100 mL of sterile 0.1% peptone water containing 0.85% NaCl and 1% Tween 80 as emulsifier at temperatures of 40-45^{o}C for 2 min in a Stomacher 400 Lab Blender. Ten mL of the preceeding dilution were mixed with 0.1% sterile peptone water. Total plate count were enumerated in standard plate count agar (Oxoid agar) after incubation at 35^{o}C for 48 h ± 2 h, then all colonies on plates were counted (Harrigan & Mc Cance, 1976).

Determination of Thiobarbituric Acid Reactive Substances (TBARS) Values

Ten grams of minced *se'i* was homogenized with 100 mL distilled water for 2 min. The homogenized sample was added some drops of 4 M HCl slowly until the mixture reached the pH of 1.5. Then the mixture was warmed and destilated (5 mL), then distilled solution was collected in conical flask. A blank was prepared with the same protocol using 100 mL distilled water without sample (Mohd-Esa *et al*., 2010). The thiobarbituric acid reactive substances (TBARS) values were expressed as mg malonaldehid/kg sample.

Residual Nitrite Analysis

Residual nitrite level of *se'i* was determined as mg $NaNO_2$/kg meat by a spectrophotometer method at 540 nm as described in AOAC (1995). For each treatment, measurements were made twice. Five grams of *se'i* were minced and transferred into a 250-mL beaker glass. Forty milliliter of water was added and the solution was heated to 80^{o}C for 15 min then transferred into a 250-mL volumetric flask. Hot water was added to bring the final volume of about 200 mL then the solution was transferred to steam bath for 2 h then the solution was shaked occasionally. The solution was cooled to room temperature. Water was added to reach the final volume of 250 mL. The solution was filtered and centrifuged to make it clear. A 2.5 mL of sulphanilamide solution was added to aliquot containing 5-50 ug $NaNO_2$ in 50 mL volume flask and the solution was mixed. Five minutes later, a 2.5 mL NED reagent was added, mixed, and diluted to a final volume of 100 ML mixed and let color develop in 15 min. A 5-mL portion of the solution was transferred to photometer cell to measure absorbance at 540 nm against blank consisted of 45 mL water, 2.5 mL of sulphanilamide reagent, and 2.5 mL of NED reagent. The concentration of nitrite was determined by comparing with standard curve with a straight line up to 1 ppm $NaNO_2$ in a final solution. Standard curve was made by adding 10, 20, 30, and 40 mL of nitrite working solution to 50 mL volume of flasks. A 2.5 mL of sulphanilamide reagent was added and after 5 min a 2.5 mL of NED reagent was added.

Organic Acids Analysis

Ascorbic acid was determined by UV-Visible Spectrophotometery (Mussa & Sharaa, 2014). Oxalic acid, malic acid, citric acid and sucinic acid was determined by high performance liquid chromatography (HPLC) (Ergonul & Nergiz, 2010). Tartaric acid was determined by high performance liquid chromatography (HPLC) (Kowalski & Wittrig, 2017).

Statistical Analysis

The data of aroma, taste, and tenderness were analyzed using non-parametric Kruskal-Wallis test. The Mann-Whitney test was used to differentiate among the means (significance P<0.05; highly significance was P<0.01). Values of L, a, and b, TPC, TBARS, and residual nitrite data were calculated with analysis of variance (ANOVA) Duncan test was used to determine the differences among means (significantly different was P<0.05) (SPSS, 20).

RESULTS

The aroma, taste, and tenderness of *se'i* marinated with roselle and lime extracts are presented in Table 1.

Table 1. Score averages of aroma, taste, and tenderness of se'i marinated with roselle and lime extracts

Treatments	Aroma	Taste	Tenderness
Control	4.82±0.01^{a}	6.99±0.01^{a}	6.50±0.01^{a}
Roselle 1%	4.71±0.02^{a}	7.03±0.02^{a}	6.62±0.01^{a}
Roselle 2%	4.77±0.01^{a}	7.00±0.01^{a}	6.72±0.02^{a}
Roselle 3%	4.76±0.01^{a}	7.81±0.01^{b}	6.97±0.01^{b}
Lime 1%	4.74±0.01^{a}	7.17±0.01^{a}	7.01±0.01^{b}
Lime 2%	4.54±0.01^{a}	7.19±0.01^{a}	6.59±0.01^{a}
Lime 3%	4.46±0.02^{a}	7.49±0.01^{b}	7.40±0.01^{c}

Note: Means in the same column with different superscripts differ significantly (P<0.05). Aroma: 1= odorless, 5= strong se'i aroma; Taste 1= extremely dislike, 9= extremely like; Tenderness: 1= extremely tough, 9= extremely tender.

Addition of roselle or lime extract inproved (P<0.05) taste and tenderness of *se'i* but did not affect the aroma of *se'i*. *Se'i* samples treated with 3% of roselle and 3% of lime had higher scores on taste compared to the other treatments. However, *se'i* samples treated with 3% of roselle, 1% and 3% of lime had higher scores of tenderness compared to other treatments. All *se'i* samples were in the rate of "*se'i* aroma".

The color analyses of *se'i* marinated with roselle and lime extracts are presented in Table 2. There was a significant interaction effect (P<0.05) between the type of extract and the level of extracts on lightness (L), redness (a), and yellowness (b) values of *se'i*. The lowest lightness values were found in *se'i* samples treated with 1% of lime and 3% of roselle extracts. The highest lightness (*L**) values were found in *se'i* samples treated with 1%-2% of roselle and 2% of lime extracts.

The addition of roselle and lime extracts decreased the redness (a) values of *se'i*. The highest redness value was found in control *se'i* (P<0.05), and *se'i* treated with 1% roselle extract had the lowest redness values (Table 2). The addition of 1% of roselle extract kept yellowness (b) values at the lowest level and the highest yellowness (b) values was found in *se'i* added with 3% of lime extract (P<0.05) (Table 2). The number of total bacteria or total plate count (TPC) (log cfu/g), thiobarbituric acid (TBARS) value (mg malonaldehid /kg), and nitrite residual (mg/kg) values of *se'i* marinated with roselle and lime extract are presented in Table 3. There was a significant interaction effect between the type of extract and concentration of extract on total plate count (TPC) (P<0.05). The type and the concentrations of the extracts had significant interaction effects on TBARS parameter. In addition, the type and the concentration of extract had interaction effects (P<0.05) on nitrite residual of *se'i.*

DISCUSSION

Sensory Evaluation

The result about aroma found in this experiment contradicted with those reports by Malelak *et al.* (2015) that aroma of *se'i* was affecteded by lime extract. This difference could be due to the lower level of the extract used in this experiment than that used in Malelak *et al.* (2015). Bozkurt & Belibagl (2008) reported that ad-

Table 2. Average of color L, a, and b values of se'i marinated with roselle and lime extracts

Variables	Source of extract	Level of extract (%)			
		0	1	2	3
L	Roselle	40.00±0.01^{b}	47.04±0.01^{c}	46.12±0.01^{c}	36.04±0.01^{a}
	Lime	40.00±0.01^{b}	36.81±0.01^{a}	44.06±0.01^{c}	38.51±0.01^{ab}
a	Roselle	18.00±0.01^{e}	12.97±0.02^{a}	16.97±0.03^{d}	16.57±0.01^{cd}
	Lime	18.00±0.01^{e}	16.66±0.01^{cd}	13.98±0.02^{b}	16.36±0.03^{c}
b	Roselle	14.00±0.01^{c}	11.32±0.02^{a}	14.09±0.01^{c}	12.09±0.03^{ab}
	Lime	14.00±0.01^{c}	12.00±0.02^{ab}	12.38±0.01^{b}	15.03±0.01^{d}

Note: Means in the same variable with different superscripts differ significantly (P<0.05).

Table 3. The number of total microbes (log cfu/g), thiobarbituric acid (TBARS) value (mg malonaldehid/kg), and nitrite residual (mg/kg) values of se'i marinated with roselle and lime extracts

Variables	Source of extract	Level of extract (%)			
		0	1	2	3
Total microbes (log cfu/g)	Roselle	1.62±0.15^{d}	1.61±0.01^{d}	0.65±0.01^{c}	0.56±0.04^{b}
	Lime	1.62±0.15^{d}	0.63±0.11^{c}	0.55±0.01^{ab}	0.48±0.04^{a}
Thiobarbituric acid reactives substances (TBARS) value (mg malonaldehide/ kg)	Roselle	0.77±0.11^{f}	0.65±0.01^{e}	0.57±0.02^{d}	0.57±0.01^{d}
	Lime	0.77±0.11^{f}	0.55±0.12^{c}	0.39±0.02^{b}	0.37±0.03^{a}
Nitrite residual value (mg/kg)	Roselle	29.00±0.15^{c}	9.00±0.10^{b}	5.00±0.01^{a}	9.00±0.02^{b}
	Lime	29.00±0.15^{c}	4.00±0.02^{a}	4.00±0.02^{a}	5.00±0.01^{a}

Note: Means in the same variable with different superscripts differ significantly (P<0.05).

dition of roselle increased the aroma score of kavurma and sucuk (Karabacak & Bozkurt, 2008). This difference could be due to the number and types of ingredients used in sucuk processing that were the mixture of black paper, cumin, and also starter cultere while in *se'i* processing only used salt and saltpeter.

Se'i added with 3% of roselle extract or 3% of lime extract had increased score taste. Karabacak & Bozkurt (2008) reported that the use of roselle increased flavor and tenderness of sucuk. Malelak *et al.* (2015) also explained that supplementation of lime extract increased taste score of *se'i*.

The samples added with 3% of lime had increased tenderness (P<0.05) compared to the other four treatments and control. It was reported that citric acid was more effective as a tenderising agent for beef and goat meats compared to the other organic acids (Iqbal *et al*., 2016). Citric acid was also reported to increase the tenderness of chicken breast (Kim *et al*., 2015).

Iqbal *et al.* (2016) suggested that acids could weaken the meat structure to improve proteolysis. Proteolysis breaks off peptide bonds of amino acids in meat proteins such as collagen that results in increasing tenderness (Marino *et al*., 2013). However, the use of citric acid did not enhance the tenderness of culled cow meat (Klinhom *et al*., 2015). The result of this experiment showed that although citric acid content in lime was lower than in roselle, addition of 2% of lime did not increase the *se'i* tenderness. Thus, other factors might be involved in affecting the tenderness of *se'i*.

Color Determination

The L values found in the present experiment were in agreement with those reported by Bozkurt & Belibagl (2009) that addition of roselle increased ligthness value of kavurma. However, Bozkurt & Belibagl (2009) found that roselle did not affect L value of sucuk. *Se'i* treated with 1%-2% of roselle extract and 2% of lime increased lightness (L) values.

It was reported that lightness values were influenced by antioxidant compounds (Fernandez-Lopez *et al*., 2005). Anthocyanins and organic acids in roselle had the ability as an antioxidant (Bozkurt & Belibag, 2009). Antioxidant compounds could retard metmyoglobin (MMb) formation that eventually decreased the lightness values (Fernandez-Lopez *et al*., 2005). For this reason, addition of 1%-2% of roselle extract increased the lightness (L) of *se'i* samples (P<0.05), however the lightness of the *se'i* was decrease when the level of rosella extract increased to 3%. The increased level of the extract used in the *se'i* processing will increase the total concentration of antioxidant.

When 1% of lime extract was added, the lightness values decreased (P<0.05). The extract could weaken the meat structure/myofibrils (Iqbal *et al*., 2016) so the water would be released from the structure and when the *se'i* was smoked the water would loss so the lightness values of the *se'i* would decrease. The lightness values increased by adding 2% of extract then decreased again at the level of three percent. The decrease in L values with the addition of 3% of the lime extract was due to the increased total antioxidant content in the extract. The decreased L values were caused by the decrese in lightness and increase in a dark color formation due to the browning reactions (Bayram & Bozkurt, 2007). However, if the L values were above 50, the meat was brighter than darker (Patrascu *et al*., 2013).

Addition of citric acid in chicken breasts reduced redness (a) values due to denaturation of myoglobin (sarcoplasmic heme protein) (Kim *et al*., 2015). The lowest a values of *se'i* treated with 1% of roselle extract in this experiment indicated that denaturation of myoglobin was occured due to the presence of citric acid and the organic acids in roselle as well as in lime extracts. Similar result was reported that the addition of roselle decreased the a value of sucuk (Bozkurt & Belibgal, 2009). It was interested that the addition of 2%-3% roselle extract increased the a values and this phenomenon was also observed in the addition of 1% and 3% of lime extract but the values were still lower than the control. It seems that the role of citric acid or other organic acids in influencing the a values was affected during smoking. It was reported that denaturation of protein increased when the internal temperature of meat during processing increased which caused the decrease in myoglobin content (Sen *et al*., 2014). However, if the meat was processed with a low temperature during a longer duration of cooking condition (< 65^{o}C) the meat would be more reddish since the process of myoglobin denaturation was low (Sanchez Del Pulgar *et al*., 2012). Other possibility that affected this phenomenon was the heating during smoking that might prevent the binding of citric acid to iron of heme in myoglobin. The prevention of iron binding with citric acid will increase the availability of heme to bind oxygen resulting in the increase in a values of *se'i*. However, further experiment is needed to support this argument.

The result about the yellowness (b) values of *se'i* found in this experiment was similar to that reported by Bozkurt & Belibagl (2009) that the addition of roselle could reduce yellowness values of kavurma. The decrease in yellowness values suggested that the color of *se'i* turned to blue rather than yellow. In this experiment addition of 1% and 3% of roselle extract and 1% and 2% of lime extract reduced the yellowness (b) values of *se'i*, but the value increased when the level of lime extract addition was increased to 3%.

Microbial Population

All treatments caused the decrease in total plate count (TPC) number, except in the addition of 1% of roselle extract. The lowest TPC numbers was found in *se'i* treated with 3% of lime (0.48 log cfu) and the highest was found in control and *se'i* treated with 1% of roselle extract i.e., 1.62 and 1.61 log cfu, respectively. TPC of *se'i* marinated in lime extract was lower than those marinated in roselle extract. The lower TPC in *se'i* marinated in lime extract could be due to the higher antibacterial content in lime extract than in roselle extract. TPC number in this experiment was lower than that was reported by Malelak *et al.* (2015). However, all *se'i* samples in all treatment and control in this experiment had number of bacteria lower than 1x10^5 cfu. Maximum bacterial contamination permited in smoked meat was 1x10^5 cfu (5 log cfu/g) (SNI.7388:2009).

Roselle and lime extracts contain organic acids (Table 4) such as citric acid that has an antibacterial activity (Hussain *et al*., 2015). The differences in the amount of organic acids content in these two extracts (Table 4) used in this experiment could explain the differences in their effects on the reducing of TPC in *se'i*.

Thiobarbituric Acid Reactive Substances (TBARS) Values

All treatments used in this experiment reduced the thiobarbituric acid (TBARS) values of *se'i*. The increase in the levels of either roselle or lime extract used in this experiment decreased the TBARS values of *se'i*. These results recommended that these two extracts inhibited lipid oxidation and the lime extract had a higher antioxidant activity than roselle extract. Fernandez-Lopez *et al* (2005) also reported that lime extract showed an antioxidant activity in beef meat ball. Roselle showed an antioxidant activity in sucuk (Karabacak & Bozkurt, 2008) and kavurma (Bozkurt & Belibagl, 2009).

Nitrite Residual Analysis

All treatments reduced the nitrite residual value and the lowest value was foud in *se'i* marinated in 2% roselle extract and 1% and 2% lime extracts. These results were similar to those reported by Malelak *et al.* (2015) that application of lime in the *se'i* production decreased nitrite residual values. However, the nitrite residual amount in this experiment was lower than that reported by Malelak *et al.* (2015). This difference was due to the amount of nitrate (saltpeter) used in this experiment was 300 mg/kg of meat that was lower than nitrate used in Malelak *et al.* (2015) that was 500 mg/kg of meat. The level of nitrate addition that is permited in meat processing products is 500 mg/kg (Indonesian Health Department 1168/1999). The mean of residual nitrite permitted in processing meat is 30 mg/kg (National Agency for Drug and Food Control, 2013). Thus, the ranges of nitrate used and the residual nitrite level in this experiment were in the ranges allowed by the government rule.

The reduced nitrite residual levels in the *se'i* processed in this experiment were related to the bioactive content of roselle and lime extracts. Viuda-Martos *et al* (2009) stated that the use of citrus co-product (orange dietary fiber) could reduce nitrite residual in dry-cured sausage and bologna sausage dry sausage meat product due to the presence of bioactive compounds such as organic acids and polyphenols. Kilic & Oztan (2013) reported that the use of organic acids (ascorbic acid) reduced nitrite residual in smoked fish. Although the ascorbic acid in roselle calyces was higher than in lime (Table 4), the nitrite residual level in *se'i* treated with lime was lower than in *se'i* treated with roselle calyces. The results of observation showed that the contents of other organic acids, except ascorbic acid, in lime extract was higher than those in roselle calyces extract (Table 4). The differences in the amount of organic acids compounds in the two extracts resulted in the differences in the nitrite residual content in *se'i* samples (Table 3).

The reduction of the nitrite residual levels could be an acceptable alternative to reduce the intake of nitrite in processed meats to avoid consumers from negative effect of health.

Table 4. Organic acids contents in roselle and lime extracts

Kind of extract	Ascorbic acid (mg/100g)	Citric acid (%)	Tartaric acid (%)	Malic acid (%)	Oxalic acid (%)	Succinic acid (%)
Roselle	106	2.025	2.167	1.657	1.890	1.761
Lime	12	4.391	4.744	7.307	-	-

CONCLUSION

The addition of 3% of roselle calyces extract and 3% of lime extract increased the score of taste and tenderness, but decreased the a values, TPC, TBARS, and nitrite residual value. The marination of *se'i* in 3% of roselle calyces extract decreased the b value but marination in 3% of lime extract increased the b value of *se'i*. Marination of *se'i* in 3% of roselle extract or 3% of lime extract gave the best effect on taste, tenderness, TPC, and TBARS values of *se'i*.

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REFERENCES

AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Ardabili, A. G., R. Farhoosh, & M. H. H. Khodaparast. 2010. Frying stability of canola oil in presence of pumpkin seed and olive oils. Eur. J. Lipid Sci. Technol. 112: 871-877. https://doi.org/10.1002/ejlt.200900257

Bayram, M. & H. Bozkurt. 2007. The use of bulgur as a meat replacement: bulgur-sucuk (a vegetarian dry-fermented sausage). J. Sci. Food Agric. 87:411-419. https://doi.org/10.1002/jsfa.2712

Boshtam, M., J. Moshtaghian, & G. Naderi. 2011. Antioxidant effects of Citrus aurantifolia (Christm) juice and peel extract on LDL oxidation. J. Res. Med. Sci 16:951-955.

Bozkurt, H. & K. B. Belibagl. 2009. Use of Rosemary and *Hibiscus sabdarifa* L in production of Kavurma, a cooked meat product. J. Sci. Food Agric. 89: 1168. https://doi.org/10.1002/jsfa.3570

Dainty, R. H., R. A. Edwards, & C. M. Hibbard. 1985. Time course of volatile compounds formation during refrigerated storage of naturally contaminated beef in air. J. Appl. Bacteriol. 59:303-309. https://doi.org/10.1111/j.1365-2672.1985.tb03324.x

Demeyer, D., K. Honikel, & S. De Smet. 2008. The World Cancer Research Fund report 2007: a challenge for the meat processing industry. Meat Sci. 80:953–9. https://doi.org/10.1016/j.meatsci.2008.06.003

El-Alim, S. S. L., A. Lugasi, J. Hovari, & E. Dworschak. 1999. Culinary herbs inhibit lipid oxidation in raw and cooked minced meat patties during storage. J. Sci. Food Agric. 79:277-285. https://doi.org/10.1002/(SICI)1097-0010(199902)79:2<277::AID-JSFA181>3.0.CO;2-S

Ergonul, P.G. & C. Nergiz. 2010. Determination of organic acids in olive fruit by HPLC. Czech. J. Food. Sci. 28:202-205

Fernandez-Lopez, J., N. Zhi., L. Aleson-Carbonell., J. A. Perez-Alvarez, & V. Kuri. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci. 69: 371-380. https://doi.org/10.1016/j.meatsci.2004.08.004

Ghafar, M. F. A., K. N. Prasad., K. K. Weng, & A. Ismail. 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from citrus species. Afr. J. Biotechnol. 9: 326-330.

Harrigan, W. F. & M. E. McCance. 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, U.K. p. 452.

Hussain, G., A. Rahman, T. Hussain, S. Uddin, & T. Ali. 2015. Citric and lactic acid effects on the growth Inhibition of *E. coli* and *S. typhymurium* on beef during storage. Sarhad Journal of Agriculture 31: 183-190. https://doi.org/10.17582/journal.sja/2015/31.3.183.190

Indonesian Health Department. 1999. Rule of Health Ministry of Indonesia. No. 1168/MENKES/PER/1999. Food additive.

Iqbal, A., O. E. Hany, A. Neelam, A. Alamgir, & S. Nawaz. 2016. In Vitro effects of papaya (Carica papaya), lemon juice (Lime), vinegar and yogurt as a meat tenderizer. J. Chem. Biol. Phys. Sci. 6(2): 568-573.

Karabacak. S. & H. Bozkurt. 2008. Effects of Urtica dioca and Hibiscus sabdarifa on the quality and safety of sucuk (Turkish dry-fermented sausage). Meat Sci. 78:288-296. https://doi.org/10.1016/j.meatsci.2007.06.013

Kilic, A. & A. Oztan. 2013. Effect of ascorbic acid utilization on cold smoked fish quality (oncorhynchus mykiss) during process and storage. Food Science and Technology Research 19:823-831. https://doi.org/10.3136/fstr.19.823

Kim, J. H., G. E. Hong, K. W. Lim, W. Park, & C.H. Lee. 2015. Influence of citric acid on the pink color and characteristics of sous vide processed chicken breasts during chill storage. Korean J. Food Sci. Anim. Resour. 2015. 35: 585–596. https://doi.org/10.5851/kosfa.2015.35.5.585

Klinhom, P., J. Klinhom, J. Senapa, & S. Methawiwat. 2015. Improving the quality of citric acid and calcium chloride marinated culled cow meat. Int. Food Res. J. 22:1410-1416.

Kowalski, J. & B. Wittrig. 2017. Simple, Reliable HPLC Analyses of Organic Acids Using Water–Compatible Allure or Ultra C18 Columns. Restek Corporation, U.S. 110 Benner Circle, Bellefonte.

Kumari, S., N. Sarmah, & A. K. Handique. 2013. Antioxidant activities of the unripen and ripen Citrus aurantifolia of assam. IJERSET 2:4811 - 4816

Mahadevan, N., Shivali, & P. Kamboj. 2009. Roselle Linn. An overview. Natural Product Radiance 8: 77-83.

Malelak, G. E. M., G. M. Sipahelut, I. G. N. Jelantik, M. R. Denoratu, & H. J. D. Lalel. 2015. Characteristics of se'i (rotenesse smoked meat) treated with coconut shell liquid smoked and lime extract. Med. Pet. 38: 89-94. https://doi.org/10.5398/medpet.2015.38.2.89

Marino, R., M. Albenzio, A. della Malva, A. Santillo, P.Loizzo, & A. Selvi. 2013. Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time. Meat Sci. 95:281-287. https://doi.org/10.1016/j.meatsci.2013.04.009

Mohd-Esa, N., F. S. Hern, A. Ismail, & C. L Yee. 2010. Antioxidant activity in different parts of roselle (Roselle L.) extracts and potential exploitation of the seeds. Food Chem. 122: 1055–1060. https://doi.org/10.1016/j.foodchem.2010.03.074

Mussa, S.B. & I.E. Sharaa. 2014. Analysis of vitamin C (ascorbic acid) contents packed fruit juice by UV-Visible Spectrophotometery and redox titration methods. IOSR Journal of Applied Physics. 6: 46-52. https://doi.org/10.9790/4861-06524652

National Agency for Drug and Food Control. 2013. Regulation of the Head of Indonesian National Agency for Drug and Food Control No. 36/2013 on the Maximum Limit of Preservative as Food Additive. Jakarta, Indonesia.

National Standardization Agency of Indonesia. 2009. Maximum permitted contaminated microbes in food. Indonesian National Standard (SNI)7388:2009.

Onibi, G.E. & I. B. Osho. 2007. Oxidative stability and bacteriological assessment of meat from broiler chickens fed diets containing Hibiscus sabdariffa calyces. African Journal of Biotechnology. 6: 2721-2726. https://doi.org/10.5897/AJB2007.000-2435

Patrascu, L., I. Dobre, & P. Alexe. 2013. Effect of thumbing time, injection rate and K-carrageenan addition on processing, textural and color characteristics of Biceps femoris muscle. Food Technol. 37: 69-84.

Sanchez del Pulgar, J., A. Gazques, & J. Ruiz-Carrascal. 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature and cooking time. Meat Sci. 90:828-835. https://doi.org/10.1016/j.meatsci.2011.11.024

Santos-Fandila, A., F. J. Camino-Sánchez, & A. Zafra-Gómez A 2014: Degradation markers in nutritional products: A review. Austin J. Anal. Pharm. Chem. 1: 1-7.

Sebranek, J. G., V. J. H. Sewalt, K. L. Robbins, & T. A. Houser. 2005. Comparison of a natural Rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 69:289-296. https://doi.org/10.1016/j.meatsci.2004.07.010

Sen, A.R., B. M. Naveena, M. Muthukumar, & S. Vaithiyanathan. 2014. Colour, myoglobin denaturation and storage stability of raw and cooked mutton chops at different end point cooking temperature. J. Food Sci. Technol. 51: 970–975. https://doi.org/10.1007/s13197-011-0557-z

Shah, M. A., S. J. D. Bosco, & S. A. Mir. 2014: Plant extracts as natural antioxidants in meat and meat products. Meat Sci. 98: 21-33. https://doi.org/10.1016/j.meatsci.2014.03.020

Viuda-Martos, M., J. Fernández-López, E. Sayas-Barbera, E. Sendra, C. Navarro, & J. A. Perez- Alvarez. 2009. Citrus co-products as technological strategy to reduce residual nitrate content in meat products. J. Food Sci. 74:93-100. https://doi.org/10.1111/j.1750-3841.2009.01334.x